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RESEARCH PAPER

Biomass and nutrients allocation in pot cultured beech seedlings: influence of nitrogen fertilizer

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Abstract: Allocation of biomass and nutrient elements including Nitrogen to above and belowground compartments of beech seedlings (*Fagus sylvatica* L.) treated by labeled nitrogen fertilizer in the form of $^{15}NH_4$ and $^{15}NO_3$ were investigated at the end of two successive growing seasons. Pot cultured beech seedlings were grown at a green house on intact soil cores sampled from three adjacent stands including beech, Norway spruce and mixed beech-spruce cultures of Solling forest, Germany. Comparing biomass allocation and nutrients concentrations of the seedlings between the control and ^{15}N-fertilized treatments revealed no significant effect of N fertilization on nutrients uptake by seedlings over the experiment. The form of N input influenced its movement into plant pools. It was demonstrated that beech seedlings take up nitrogen mainly in the form of nitrate, which is then reduced in the leaves, although the differences between the retention of NO_3^--N and NH_4^+-N in plants were not statistically significant. Percent recoveries of ^{15}N in trees were typically greater after $^{15}NO_3$ than after $^{15}NH_4$ additions. It was indicated that immobilization of ^{15}N tracer in fine roots was a slower process comparing other plant compartments such as stem and coarse roots, but a powerful sink for N during the course of study.

Keywords: beech seedling; nitrogen fertilizer; biomass; nitrogen immobilization; nutrient; mycorrhiza.

Introduction

Temperate forests are recipients of anthropogenic nitrogen deposition. Because growth in these ecosystems is often limited by N availability, chronic N inputs from the atmosphere during recent decades has resulted in a greater availability of N in forest ecosystems and an imbalance of plant mineral nutrients (Van Dijk and Roelofs 1988). Many studies have reported forest responses to N deposition ranging from changes in plant tissue chemistry, small nitrogen exports to tree tissue, nutrients imbalances, tree growth declines, and large nitrate exports to drainage water (e.g., Aber et al. 1993; Nilsson and Wiklund 1994; Boxman et al. 1995; Bredemeier et al. 1995; Gundersen and Rasmussen 1995; Wright and Tietema 1995; Wright et al. 1995). Descriptive information on C and N allocation in different tree species under changing environmental conditions is widely available (Canham et al. 1996), but there is little quantitative knowledge of nutrients allocation in seedlings due to elevated atmospheric N-input. Nitrogen fertilization experiments with beech in early stages of growth are few and the effects of N fertilizer on growth and biomass production are inconsistent and uncertain (Kenk and Fischer 1988). Information on the effect of N over supply in the form of N fertilizer on carbon, nitrogen and mineral nutrients uptake and partitioning is necessary to understand and predict seedlings growth and development under chronic nitrogen input conditions. Application and recovery of labelled nitrogen tracers in forests has proven to be a powerful tool for gaining insight into N fluxes and transformations in the soils (Davidson et al 1990; Tietema and Wessel 1992) and vegetation (Preston et al. 1990). In a few manipulations of N inputs to forests, ^{15}N has been added as a tracer to study altered fates and redistributions of NH_4^+ and NO_3^- at the ecosystem level (Nadelhoffer and fry 1994; Nadelhoffer et al. 1995 and 1999; Tietema et al. 1998). Tracing the two N mineral forms is advantageous because the system's natural N level is relatively unaltered.

The aim of the present study was: (1) to identify the effect of N input on biomass allocation and nutrient elements distribution between the above and belowground compartments of the beech seedlings, (2) to investigate the retention and recoveries of $^{15}NH_4$ and $^{15}NO_3$ tracers to assess how forms of N input affect the partitioning of nitrogen deposition into different compartments of the beech seedlings. To quantify N form immobilization at early stages of plant growth $^{15}NH_4$ and $^{15}NO_3$ fertilizers were applied separately for two consecutive growing seasons in through fall of the pot cultured beech seedlings grown on intact forest soil samples of the beech, spruce and mixed species stands.

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Materials and methods

Site description

The Study site is located at the Solling forest about 70 to 80 km southward Hannover, Lower Saxony, Germany (51°47′N and 9°37′E) on slightly inclined (2–4°) slopes. The area is situated at 500 m elevation a.s.l., with a mean annual air temperature of 6.5°C and an annual precipitation of 1050 mm. The temperatures range from an average of 14°C in July to -2°C in January. The dominant soil type are podsolic, slightly pseudogleyic Dystric Cambisol (FAO) developed on triassic sandstone covered by a layer of loess with a thickness varying from 0.2 to 2 m (average 80 cm) (Tiktak et al. 1995). Soil texture is dominated by silty loam. Morphological humus forms are typical moder. Three adjacent stands were chosen for the study: a mature (100–120 years) Norway spruce (*Picea abies* L.karst.) stand partly covered by grass, a 100–120-yr-old beech (*Fagus sylvatica* L.) stand and a mixed spruce-beech stand covered by 100–120-yr-old trees.

Soil sampling and experimental design

In the August of first growing season, some one hundred twenty intact soil cores (forest floor plus two cm mineral soil) were taken randomly from each stand (n = 40) using PVC cylindrical pots (15.2 cm in diameter, 19 cm in depth). All samples were transferred to a green house and partitioned into 4 wagons (250 cm length, 92 cm across, 26 cm depth) with 10 replicates from soil samples of each stand at each one. The leachate from each sample passed through 10 cm quartz sand in a PVC cylinder set at the bottom of each pot, conducted the leachates through a silicon tube to a brown glass bottle. A cooling system was installed; circulating cool liquid permanently through a flexible tube spiraled four times around each pot to hold the temperature of the samples constant. The sides and the bottom of each wagon were isolated with a 4-cm thick Styropor. The empty space between the pots at each wagon filled with insulator materials to avoid losses of temperature. The air and soil temperature were continuously recorded at 5 cm on top of the forest floor samples and at 3 cm depth (Fig. 1).

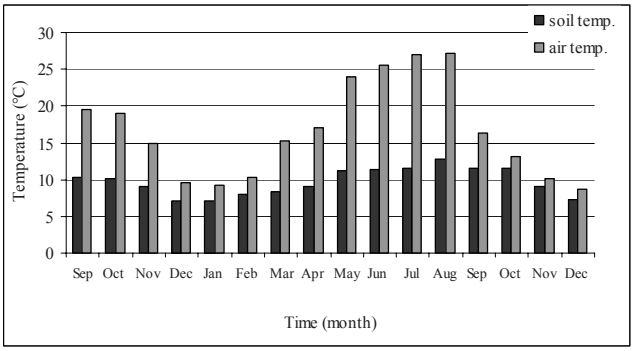

Fig. 1 Mean air and soil temperatures (°C) during the incubation experiment

The samples were artificially irrigated every two days using a bore hole PVC cap (5 cm height) contained small nylon threads which came through the fine holes. The pH and the applied amounts of cations and anions in throughfall were calculated on the basis of the long term annual input at the study area (Matzner 1989). The element concentrations in the throughfall solution were constant during the experiment. The leachates volume of the soil solutions were collected in 4 weeks intervals and stored at 4°C for further processing.

Treatments

Some six beech seeds were planted in depth of 0.5 cm in the pots (n=60) at the end of August, which germinated after about three weeks. The control treatment (n=36) consisted of plants and non-plants samples, each with 18 replicates, received no nitrogen input in throughfall. The N-fertilized treatments (n=84) were divided in plants and non-plants samples, each with 42 replicates. Nitrogen fertilizer was supplied with either 1.17 $mg \cdot L^{-1}$ NO_3-N as 10% enriched $NH_4{}^{15}NO_3$, or with 1.18 $mg \cdot L^{-1}$ NH_4-N as 10% enriched $^{15}NH_4NO_3$. In this treatment half of the plants and non-plants samples received nitrogen as ^{15}N-nitrate and half of them as ^{15}N-ammonium. The nitrogen input was in magnitude at the same rate of throughfall N input at Solling forest amounted to 1.59 $kg \cdot ha^{-1} \cdot a^{-1}$. The total throughfall volume added up to 816 mm and the amount of nitrogen input in ^{15}N-fertilized treatment over the course of experiment accounted for 1.92 $g \cdot m^{-2}$.

Chemical analyses of plant and soil

Seed leaves and the leaf litter samples of the first vegetation period were taken from the shoot tips 3 months after bud break at December. Leaf litter samples of the second vegetation year were collected at mid November and the whole seedlings were harvested at mid December second year. Plant compartments were divided into seed leaves, leaf litter of the first and second growing seasons, stem, coarse roots (≥3 mm diameter) and fine roots (<3 mm diameter) which involved those in forest soil containing mycorrhizal and non mycorrhizal root tips and the fine roots grew in sand layer underneath. The root system partitioned by size and depth in order to represent some trends in biomass, nutrient concentrations and N distribution between the below-ground biomass compartments. Roots were separated from soil by washing and sieving method for several times. All tree biomass compartments were dried at 65°C for 96 h, milled, homogenized and weighed. Stems and coarse roots were milled in a planetary mill (Retsch, Hann, Germany), the other plant components ground in a piston-action ball mill. The mineral nutrients Phosphorous, Sulphur, Potassium, Calcium, Magnesium, and Manganese in plant compartments were analyzed by ICP-AES (Spectro Analytical Instruments, Kleve, Germany) after pressure digestion in 65% concentrated HNO_3. Carbon, Nitrogen and nutrient cations and anions concentrations were calculated on a mass basis and were expressed as a percentage of dry mass. The biomass content of each compartment was measured on a mass basis per seedling and the nutrient uptake from soil expressed as

mg nutrient per square meter. Organic carbon and nitrogen concentrations of initial soil samples were analysed by dry combustion with a CN- auto analyser (Vario, Elementar Analysensysteme, Hanau, Germany). Soil pH was measured with a digital pH-meter (WTW GmbH Wesl-Germany) in water and 1 mol·L^{-1} KCl.

^{15}N mass measurements

The ^{14}N/^{15}N isotope measurements were made with an isotope ratio mass spectrometer (Delta Plus, Finnigan Mat GmbH, Bremen, Germany) coupled to an elemental analyzer (EA 1108, Fisons, Rodano, Milan, Italy) in online mode. ^{15}N abundance is expressed in atom percent by the ratio:

$$\text{Atom}\,\%^{15}N = \frac{^{15}N}{^{14}N + ^{15}N} \times 100$$

The values of ^{15}N enrichment (atom %^{15}N excess) were calculated by subtracting ^{15}N values from ^{15}N natural abundance of the control. For determination of ^{15}N concentrations of soil and plant materials total N content and the biomass of the respective parts were taken into consideration.

Total ^{15}N content = (^{15}Nt – ^{15}Nc) × [N] × plant biomass

where $^{15}N_t$ and $^{15}N_c$ are the atom % concentrations of ^{15}N in treated and control plants, respectively, [N] is the total N concentration (of a g dry weight) of plant compartment. For estimation of ^{15}N uptake per plant, the ^{15}N concentrations of above and belowground parts were summed up. Tracers recovered in pools were expressed as proportions of total ^{15}N tracer applied over the course of experiment.

%^{15}N recovery =

$$\frac{[N_{\text{pool}} \times (\text{atom}\%^{15}N_{\text{pool}} - \text{atom}\%^{15}N_{\text{control}}) \times m_{\text{pool}}]}{[N_{\text{applied}} \times (\text{atom}\%^{15}N_{\text{tracer}} - \text{atom}\%^{15}N_{ref.})] \times \text{irrigationvolume}} \times 100$$

Where, $^{15}N_{recovery}$ is the percent ^{15}N tracer recovered in the labeled N pool (%). N_{pool} is the concentration of total N in the labeled N pool (mg g^{-1}), atom %$^{15}N_{pool}$ is atom percent ^{15}N in the labeled N pool, atom %$^{15}N_{control}$ is atom percent ^{15}N in the control (non-labeled) N pool, m_{pool} is N mass of the labeled N pool (g), $N_{applied}$ is concentration of the fraction (NO_3-N or NH_4-N) in irrigated water (mg l^{-1}), atom %$^{15}N_{tracer}$ is atom percent of the applied tracer, atom %$^{15}N_{ref.}$ is atom percent ^{15}N in the reference (non-labeled) N pool and irrigation volume is the total throughfall.

Statistical analyses

The Mann-Whitney U-Test at p<0.05 level, was used to evaluate the significance of differences between the treatments in individual plant compartments, performed by the program Statistica version 6.0.

Results and discussion

Soil chemical characteristics

Site variation effects on chemical characteristics of soils were negligible between the stands (p<0.05), hence soil samples were distributed equally between the cultured control and ^{15}N-fertilized treatments (Table 1). The average soil depth ranged from 6.67 to 6.36 cm in cultured control and N-fertilized treatments. All soils were acidic with $pH_{(KCl)}$ ranging from 2.98 to 3.02 in cultured control and ^{15}N-fertilized treatments. The concentrations of organic carbon and nitrogen varied between 172–159 g·kg^{-1} and 8.83–8.08 g·kg^{-1} in cultured control and ^{15}N-fertilized treatments, respectively. The C/N ratio of forest soils ranged from 19.2 to 20.1 in cultured control and ^{15}N-fertilized treatments. The mean concentrations of nutrient elements P, S, Na, K, Ca, Mg, Mn, Fe and Al in soils of the cultured control and ^{15}N-fertilized treatments were not statistically significant (p<0.05) as shown in Table 2.

Table 1. Mean soil characteristics in cultured treatments

Treatment	depth^{ns} (cm)	mass^{ns} (Mg·ha^{-1})	pH^{ns} (H_2O)	pH^{ns} (KCl)	C_{org}^{ns} (g·kg^{-1})	N_{org}^{ns} (g·kg^{-1})	C/N^{ns}
Control	6.67	242	3.69	2.98	172	8.83	19.2
	(0.83)	(76.0)	(0.34)	(0.43)	(60.8)	(2.71)	(1.63)
^{15}N-fertilized	6.36	231	3.71	3.02	159	8.08	20.1
	(1.18)	(74.8)	(0.38)	(0.39)	(58.2)	(2.60)	(1.87)

Note: Standard deviation is given in parentheses (Values are not statistically significant at p<0.05 between the treatments)

Table 2. Mean soil element concentrations in cultured treatments

Treatment	Nutrient concentration (%)								
	P^{ns}	S^{ns}	Na^{ns}	K^{ns}	Ca^{ns}	Mg^{ns}	Mn^{ns}	Fe^{ns}	Al^{ns}
Control	0.068	0.103	0.024	0.456	0.191	0.157	0.025	1.157	1.872
	(0.012)	(0.032)	(0.000)	(0.154)	(0.106)	(0.043)	(0.010)	(0.239)	(0.502)
^{15}N-fertilized	0.070	0.096	0.028	0.525	0.217	0.180	0.029	1.259	2.096
	(0.015)	(0.033)	(0.001)	(0.179)	(0.116)	(0.048)	(0.013)	(0.263)	(0.515)

Note: Standard deviation is given in parentheses (Values are not statistically significant at p<0.05 between the treatments)

Above and belowground plant biomass

Seeds with 357-mg dry weight biomass comprised 28.6%–30.7% of the total seedling biomass (Table 3). In comparison to initial biomass of seeds, stems with 495–454-mg biomass per Rahman plant in the control and ^{15}N-fertilized treatments accounted for 39.7%–39.0% of the total seedling biomass. Other major biomass fractions were coarse roots, leaf litter of the second vegetation period, leaf litter of the first growing season, fine roots in sand layer, seed leaves, buds and fine roots in soil, respectively. Total seedling biomass ranged from 1 248 mg at the control to 1 163 mg at ^{15}N-fertilized treatment (Table 3). The total biomass content of the aboveground compartments ranged from 805 mg at the control to 803 mg at ^{15}N-fertilized treatment indicated no significant differences between the same aboveground compart-

ments of the seedlings in both treatments. The amounts of belowground biomass varied between 443 and 360 mg by control and ^{15}N-fertilized treatments, respectively. Analyses of variance (p<0.05) revealed no significant differences in biomass content of fine roots between the control and ^{15}N-fertilized treatments, while the biomass content of coarse roots were significantly decreased at ^{15}N-fertilized treatment. Shoot weight ratio (SWR) accounted for 64.4% to 68.7%, while root weight ratio (RWR) contributed to 35.6%–31.3% of the total seedling biomass between the control and ^{15}N-fertilized treatments. Nitrogen fertilizer affected the beech seedlings by stimulating growth and reducing biomass allocation to the roots. Such a growth increase and shift in biomass allocation from roots to shoots is commonly associated with increased nitrogen availability (Cannell 1985, Evans 1989). Dohrenbush (1990) and Van Hess (1997) found the belowground biomass distribution in seedlings and small saplings of pedunculate oak (*Quercus robur*) and beech (*Fagus silvatica*) comprised 45%–65% of the total biomass, respectively. In consistent with our results Ljungström and Stjernquist (1995) found a total dry weight biomass of 614 to 2 746 mg after two successive growing seasons. Glatzel and Kazda (1985) found a dry weight biomass of about 1 800 and 2 400 mg respectively in pot cultured beech seedlings transplanted from the field in the autumn of their first season and harvested in the autumn of the following year.

Table 3. Total biomass content per seedling, the percentage of the biomass allocation in different compartments to total seedling biomass and the root-to-shoot ratio at control and ^{15}N-fertilized treatments

Pool	Plant biomass			
	control		^{15}N-fertilized	
	(mg)	(%)	(mg)	(%)
seed	357	28.6	357	30.7
seed leaves^{ns}	74.4 (15.4)	5.96	79.7 (23.8)	6.85
buds^{ns}	69.4 (26.0)	5.57	73.2 (39.2)	6.29
leaf litter^{ns} (1st year)	89.1 (46.0)	7.14	106 (45.2)	9.13
leaf litter^{ns} (2nd year)	139 (113)	11.1	157 (75.5)	13.5
stems^{ns}	495 (172)	39.7	454 (23.6)	39.0
shoot weight ratio^{ns}	805 (265)	64.4	803 (310)	68.7
coarse roots^{ns}	310 (116)	24.8	238 (112)	20.5
fine roots in soil^{ns}	55.6 (36.1)	4.46	55.5 (27.5)	4.77
fine roots in sand^{ns}	77.6 (38.4)	6.22	66.6 (45.5)	5.73
root weight ratio^{ns}	443 (150)	35.6	360 (136)	31.3
plant^{ns} $_{(total)}$	1248 (385)	100	1163 (427)	100
root-to-shoot^{ns} (mg·mg^{-1})	0.63		0.53	

Note: Standard deviation is given in parentheses (Values are not statistically significant at p<0.05 between the treatments)

The ratio of root-to-shoot at the end of the second growing season increased by reference plants at the control in compare to ^{15}N-fertilized plants, varied between 0.63 and 0.53 mg·mg^{-1}. The observed difference in the balance between root-to-shoot ratio are in agreement with the review by Skärby et al (1998), where increased retention of carbon in the leaf litter of the seedlings at ^{15}N-fertilized treatment decreased allocation of carbon to roots system, leads to decreased pools of carbohydrates in roots, resulting in a lower root-to-shoot ratio. In consistent with Canham et al., (1996) and Xu and Timmer (1999) the lower values of root-to-shoot ratio in ^{15}N-fertilized treatment compared to control can be attributed to increased nutrients sink activity in shoot compartments and a shift in the functional balance between carbon and nutrient acquisition and biomass distribution by above- and belowground plant compartments.

Carbon and nitrogen stocks in plants

Compared to above and belowground compartments seeds contained the largest pool of nutrients reserves for initial growth of seedlings (Table 4). The partitioning of total carbon concentration in the aboveground compartments revealed that stems were the largest carbon concentration pool followed by leaf litter of the second and first growing seasons, seed leaves and buds. In belowground compartments total fine roots had greater C concentrations than coarse roots at both control and ^{15}N-fertilized treatments. There were no major differences in carbon concentration between the different seedlings compartments as would be expected, since carbon constitutes about half of the plant biomass (Runion et al. 1999). Seed leaves indicated the largest pool of nitrogen concentration between the aboveground compartments, followed by buds, stems and the leaf litter of the first and second vegetation period, respectively. In belowground compartments fine roots in consistent with their physiological activity and protein storage (Landsberg and Gower 1997), exhibited the second largest nitrogen concentrations pool in seedlings. Fine roots in sand layer contained the largest values of nitrogen concentration compared to fine roots in soil and coarse roots at both control and ^{15}N-fertilized treatments. The concentrations of either carbon or nitrogen in each plant compartment were not significantly altered between the control and ^{15}N-fertilized treatments. In aboveground compartments stems due to greatest biomass content indicated substantially higher N-immobilization for throughfall nitrogen input, while the leaf litter of the first and second vegetation season due to dilution effect contributed to lower values of immobilized nitrogen.

Other nutrients stocks in plants

The concentration values of nutrient cations and anions in most aboveground compartments were greatest for Ca followed by K, Mg, Mn, S and P in both treatments. In aboveground compartments the largest concentrations of Ca and Mn were observed in the leaf litter of the first vegetation period. The largest concentrations of S, K and Mg were detected in seed leaves, while the largest concentration for P was found in the buds of the beech seedlings (Table 4). In belowground compartments the largest concentrations were observed for K followed by Ca, P, S, Mg and Mn, respectively. Coarse roots contained the largest concentrations of Ca, Mg and Mn, while fine roots in sand layer were detected as the largest pool for P, S and K concentrations between the belowground compartments of the beech seedlings. Analyses of variance (p<0.05) revealed no significant effect of nitrogen fertilizer on concentration of each nutrient cation or anion in the same compartments of plants between the treatments. The results obtained in our study are in magnitude in consistent

with the values observed by Ljungström and Stjernquist (1995) for beech seedlings harvested in September, while the foliage litter concentrations of total N, P, K and Ca were small lower, the values for S and Mg were in the same level and the concentration of Mn which reflects the local site conditions were observed to be small higher than foliage concentrations reported by above mentioned authors. Compared to other plant compartments the leaf litter total nitrogen and phosphorus concentrations decreased significantly in autumn of the first and second vegetation year in both treatments. A decrease in the leaf litter mobile nutrients N, P, S and K can be related to nutrients re-translocation processes over the senescence period from leaves to storage organs due to dilution effect. Tyrrell and Boerner (1987) attributed a decrease in absolute amounts of elements in the leaves during the senescence period to resumption of these nutrients from the leaves towards perennial tissues. The N senescence could result from increased transport of leaf-derived N to woody storage tissues such as stems as observed also for adult beech trees (Gessler et al. 1998) and in roots which also contribute to N storage in young broad leaf trees (Millard and Proe 1991). A similar trend in nutrient depletion was also observed in the foliage K concentration, although the differences between the concentration of K in the foliage and stems were not statistically different. Comparing the nutrients status based on concentrations alone is not a sound basis for estimating re-translocation of nutrients (Nambiar and Fife 1991). Confirmative evidence in this regard was obtained by comparing changes in Ca/N and Ca/P ratios. Because Ca is immobile in live tissues, its ratio with N and P would reflect the loss in tissue mass likely to have occurred during leaf senescence. In the present study the ratios of Ca/N in the senesced foliage of the second season comprised 1.43–1.37 compared to corresponding values of 0.36–0.36 and 0.15–0.13 for stems and coarse roots. Similarly, Ca/P ratios accounted for 28.2–24.5 in senesced foliage of the second season, compared to 4.42–4.68 for stems and 1.74–1.57 for coarse roots, between the control and N-fertilized treatments, respectively.

Table 4. Nutrients concentrations in seeds, above- and belowground compartments of the beech seedlings in control treatment and ^{15}N-fertilized treatment

control treatment	Nutrient concentration (%DW)							
pool	C	N	P	S	K	Ca	Mg	Mn
seeds	57.3^{c}(0.14)	3.13^{c}(0.43)	0.259^{c}(0.017)	0.182^{b}(0.012)	0.719^{c}(0.033)	0.603^{ab}(0.030)	0.176^{ab}(0.005)	0.156^{ab}(0.007)
buds	45.2^{a}(0.97)	1.17^{ab}(0.14)	0.130^{b}(0.016)	0.090^{a}(0.008)	0.428^{ab}(0.040)	0.542^{ab}(0.052)	0.110^{ab}(0.028)	0.207^{ab}(0.071)
seed leaves	47.2^{ab}(0.16)	1.77^{b}(0.06)	0.102^{ab}(0.021)	0.171^{b}(0.005)	0.447^{b}(0.023)	1.205^{b}(0.035)	0.378^{b}(0.015)	0.397^{ab}(0.012)
leaf litter (1st year)	44.2^{a}(1.11)	0.88^{a}(0.12)	0.083^{a}(0.025)	0.132^{ab}(0.018)	0.324^{ab}(0.092)	1.229^{b}(0.220)	0.263^{ab}(0.088)	0.521^{b}(0.187)
leaf litter (2nd year)	47.6^{ab}(1.46)	0.69^{a}(0.06)	0.035^{a}(0.011)	0.085^{a}(0.010)	0.289^{a}(0.108)	0.987^{ab}(0.253)	0.118^{ab}(0.112)	0.484^{b}(0.221)
stems	48.7^{ab}(0.71)	1.12^{ab}(0.21)	0.091^{ab}(0.026)	0.081^{a}(0.012)	0.305^{a}(0.033)	0.403^{ab}(0.070)	0.075^{a}(0.026)	0.166^{ab}(0.059)
coarse roots	47.4^{ab}(0.70)	1.32^{ab}(0.28)	0.117^{ab}(0.028)	0.092^{a}(0.016)	0.405^{ab}(0.047)	0.203^{a}(0.043)	0.097^{a}(0.020)	0.083^{a}(0.030)
fine roots in soil	49.4^{b}(1.21)	1.57^{ab}(0.20)	0.127^{b}(0.029)	0.138^{ab}(0.028)	0.252^{a}(0.094)	0.191^{a}(0.067)	0.070^{a}(0.039)	0.040^{a}(0.016)
fine roots in sand	46.1^{ab}(1.98)	1.45^{ab}(0.26)	0.120^{ab}(0.031)	0.130^{ab}(0.023)	0.438^{ab}(0.092)	0.127^{a}(0.051)	0.077^{a}(0.022)	0.031^{a}(0.006)
^{15}N-fertilized treatment pool	Nutrient concentration (%DW)							
	C	N	P	S	K	Ca	Mg	Mn
seeds	57.3^{c}(0.14)	3.13^{c}(0.43)	0.259^{c}(0.017)	0.182^{b}(0.012)	0.719^{c}(0.033)	0.603^{ab}(0.030)	0.176^{ab}(0.005)	0.156^{ab}(0.007)
buds	45.6^{a}(0.84)	1.14^{ab}(0.12)	0.133^{b}(0.020)	0.091^{a}(0.010)	0.408^{ab}(0.047)	0.549^{ab}(0.068)	0.114ab(0.025)	0.214^{ab}(0.079)
seed leaves	46.9^{ab}(0.17)	2.02^{b}(0.05)	0.135^{b}(0.020)	0.188^{b}(0.004)	0.492^{b}(0.024)	1.154^{b}(0.036)	0.357^{b}(0.014)	0.381^{ab}(0.011)
leaf litter(1st year)	44.7^{a}(1.13)	0.94^{a}(0.16)	0.091^{ab}(0.036)	0.132^{ab}(0.018)	0.329^{ab}(0.096)	1.288^{b}(0.336)	0.289^{ab}(0.108)	0.483^{b}(0.207)
leaf litter (2nd year)	48.1^{ab}(2.08)	0.73^{a}(0.09)	0.041^{a}(0.018)	0.091^{a}(0.009)	0.303^{a}(0.127)	0.999^{ab}(0.302)	0.131^{ab}(0.087)	0.483^{b}(0.266)
stems	48.5^{ab}(0.75)	1.21^{ab}(0.17)	0.094^{ab}(0.026)	0.085^{a}(0.012)	0.307^{a}(0.036)	0.439^{ab}(0.107)	0.082^{a}(0.030)	0.167^{ab}(0.057)
coarse roots	47.3^{ab}(0.85)	1.44^{ab}(0.23)	0.121^{ab}(0.032)	0.099^{a}(0.015)	0.410^{ab}(0.052)	0.190a(0.037)	0.100a(0.028)	0.076^{a}(0.024)
fine roots in soil	49.5^{b}(1.64)	1.46^{ab}(0.18)	0.115^{ab}(0.030)	0.129^{ab}(0.025)	0.227^{a}(0.112)	0.239^{a}(0.096)	0.069^{a}(0.043)	0.036^{a}(0.013)
fine roots in sand	46.5^{ab}(2.55)	1.70^{ab}(0.31)	0.131^{ab}(0.034)	0.158^{ab}(0.064)	0.475^{ab}(0.356)	0.206^{a}(0.432)	0.095^{a}(0.071)	0.034^{a}(0.024)

Note: Standard deviation is given in parentheses (Values with different superscript letter(s) are significantly different from one another at $p<0.05$).

The results of the Ca/N and Ca/P ratios indicated more allocation of re-translocated nitrogen and phosphorus from foliage litter at the end of the season mainly to coarse roots. In comparison to other plant compartments no depletion effect were detected from the leaf litter concentrations of S as well as mainly immobile elements Ca, Mg and Mn. The large concentrations of foliage Mn in beech seedlings have also been reported by Glatzel and Kazda (1985) and in silver fir seedlings by Szymura (2003), as well as in beech trees by Zech et al. (1985). Higher leaf litter concentration of Mn in the present study has been moderated by the larger presence of Ca and Mg in leaves, which may confirm a protective function of Ca and Mg for heavy metal toxicity in foliage components.

Nutrients balance in plants

The assimilated carbon and the nitrogen uptake from soil supply by seedlings in the control and ^{15}N-fertilized treatments varied between 148–142 g·m^{-2} (C) and 1.36–1.32 g·m^{-2} (N) revealed no significant effect of N additions on the balance stocks of carbon and nitrogen in beech seedlings between the treatments (Table 5). Comparing the net nutrients uptake between the control and ^{15}N-fertilized treatments have shown a positive correlation between the forest floor nutrient availability and re-translocation in seedlings independent from N input effect. Total carbon assimilated by seedlings over the two growing seasons in both treatments was 1.94 to 1.86 times greater than the reserved carbon stocks in seeds. In contrast seedlings took up 32.6%–31.7% of

the N demand and 31.8%–32.9% of P from the forest floor and the rest provided from seed nitrogen and phosphorus supply (Table 6). This finding indicated that seeds contribute substantially in N and P nutrition of the beech seedlings. The percent of net nutrients uptake to seed nutrient reserves exhibited an imbalance for Mn (369%–380%), Ca (195%–219%) and Mg (134%–149%), inferring high accumulation of these cations in different compartments of the seedlings, however the lack of established break points precluded distinguishing between luxury or excess consumption of Mn, Ca and Mg in both control and N-fertilized treatments.

Table 5. Net nutrients uptake (nutrients balance) in beech seedlings at the end of experiment in control and ^{15}N-fertilized treatments

Treatment	Nutrient balance (g m^{-2})							
	C^{ns}	N^{ns}	P^{ns}	S^{ns}	K^{ns}	Ca^{ns}	Mg^{ns}	Mn^{ns}
control	148.4	1.36	0.11	0.22	0.69	1.56	0.31	0.77
	(1.84)	(0.76)	(0.06)	(0.03)	(0.12)	(0.21)	(0.08)	(0.17)
^{15}N-fertilized	141.8	1.32	0.11	0.24	0.65	1.76	0.35	0.79
	(2.12)	(0.68)	(0.06)	(0.04)	(0.15)	(0.31)	(0.09)	(0.20)

Note: Standard deviation is given in parentheses (Values are not statistically significant at $p<0.05$ between the treatments)

Table6. Ratio of nutrients uptake to nutrients reserves in seeds in the control and ^{15}N-fertilized plants treatments at the end of experiment.

Treatment	Nutrient uptake / seed nutrient reserve (%)							
	C	N	P	S	K	Ca	Mg	Mn
control	194	32.6	31.8	91.3	71.7	195	134	369
^{15}N-fertilized	186	31.7	32.9	98.2	68.1	219	149	380

^{15}N tracer enrichment and retention in plants

Comparing N form enrichment by mycorrhizas in mycorrhizal root tips separated from fine roots in soil exhibited similar pattern of ammonium and nitrate assimilation by mycorrhizal fungus, while the above- and belowground compartments of seedlings preferably assimilated nitrate than ammonium (Table 7).

Table 7. Percent of ^{15}N-tracer enrichment after $^{15}NO_3^-$ and $^{15}NH_4^+$ in above- and belowground compartments of the beech seedlings

pool	^{15}N-tracer enrichment (%)	
	$^{15}NO_3^-$	$^{15}NH_4^+$
buds	0.31 (0.06)	0.28 (0.07)
seed leaves	0.03 (0.00)	0.03 (0.00)
leaf litter (1st yr)	0.07 (0.01)	0.06 (0.01)
leaf litter (2nd yr)	0.19 (0.03)	0.20 (0.04)
stems	0.34 (0.12)	0.29 (0.07)
coarse roots	0.47 (0.16)	0.34 (0.08)
fine roots in forest soil	0.25 (0.21)	0.23 (0.09)
fine roots in sand layer	0.78 (0.27)	0.58 (0.14)
mycorrhizal root tips	0.30 (0.09)	0.30 (0.10)
non-mycorrhizal root tips	0.36 (0.12)	0.35 (0.15)

Note: Standard deviation is given in parentheses

The percent of tracer enrichment in mycorrhizal root tips after $^{15}NH_4^+$ and $^{15}NO_3^-$ (0.30%–0.30%) corresponds well with those in non-mycorrhizal root tips (0.35%–0.36%), revealed that the assimilated nitrogen through mycorrhizas due to physiologically inactive conditions of plants and mycorrhizas in non-vegetation period have been transited from mycorrhizal root tips into the other parts of root system. The frequency of ectomycorrhizal colonization on fine roots in forest soil by ^{15}N-fertilizer application were about one-third of that in control plants, exhibited the inhibitory effect of nitrogen application on fungal development due to reduced carbon flow from host to fungus and the resulted carbohydrate deficiency for fungal assimilations. In consistent with our results Wallander and Nylund (1991, 1992); and Tietema (1998) demonstrated that the fungal biomass of mycorrhizal seedlings has consistently been reduced at elevated levels of N supply. The partitioning of both ^{15}N tracers retained in beech seedlings followed the same pattern of total N distribution in plants. Among the aboveground compartments stems with 39% of the total seedlings biomass comprised 0.035–0.041 mg·g^{-1} of ^{15}N tracer retained after $^{15}NH_4^+$ and $^{15}NO_3^-$ inputs (Table 8). The larger amounts of ^{15}N tracer immobilization in stems compared to other plant compartments may be attributed to direct uptake or absorption from throughfall solution. In addition, nitrogen uptake by the foliage and re-translocation during the senescence period can also be involved in the process. Comparing the values of ^{15}N tracer retained after $^{15}NH_4^+$ and $^{15}NO_3^-$ inputs between the leaf litter of the first (0.006–0.007 mg·g^{-1}) and second growing seasons (0.015–0.013 mg·g^{-1}) revealed that the perennial species of beech at first growing season rely to some extent on tissue reserves for their N nutrition. The least values of ^{15}N tracer retained after $^{15}NH_4^+$ and $^{15}NO_3^-$ inputs in aboveground compartments were observed in buds (0.031–0.037 mg·g^{-1}) and seed leaves (0.006–0.006 mg·g^{-1}), where they contribute to 6.29% and 6.85% of total seedlings biomass, respectively. Comparing the below ground compartments coarse roots due to a larger pool size with 20.5% of the total seedling biomass production comprised 0.050–0.065 mg·g^{-1} of ^{15}N tracer retention after $^{15}NH_4^+$ and $^{15}NO_3^-$ inputs (Table 8). The observed values of ^{15}N tracer immobilization in roots can be attributed to direct N uptake and to some extent foliage litter re-translocation of nitrogen products at the end of the season. Fine roots in soil and sand layer underneath accounted for 0.034–0.037 and 0.096–0.130 mg·g^{-1} of ^{15}N tracer uptake after $^{15}NH_4^+$ and $^{15}NO_3^-$ inputs, although they comprised only 4.77%–5.73% of the total plant biomass. In consistent with Nadelhoffer et al. (2002) the differences between total fine roots biomass in fertilized and non-fertilized treatments (10.5 vs. 10.7%) were not statistically significant ($p<0.05$), suggest that fine root turnover and production either, do not vary or that they tend to decrease as N availability increases. In agreement with Magill et al. (1997) the concentration of ^{15}N tracer retained in fine roots can account for a substantial fraction of N immobilization in the plants. Immobilization of deposited labeled nitrogen by mycorrhizal fine roots may increase the ^{15}N sequestration by their turnover which may be detectable in the soil organic matter. A higher ^{15}N incorporation in forest soils with beech seedlings indicated that plants may have improved the N immobilization. The total amount of ^{15}N tracer retained in seedlings were lower after $^{15}NH_4^+$ than after $^{15}NO_3^-$, suggests that nitrate uptake and deposition could have a greater proportion on seedlings growth than ammonium uptake and deposition.

Nitrate may be assimilated by the plants by high energy costs involved in nitrate reduction in fine roots, or can utilize extra reductant from the light reactions of photosynthesis to reduce nitrate in foliage (Nadelhoffer et al. 1984). Most of the ^{15}N taken up by seedlings was used to build up the above ground compartments (50.3% of total ^{15}N uptake) and coarse roots (29.2% of total ^{15}N uptake), while total fine roots accounted for 20.5% of total ^{15}N uptake by plants (Table 8). The ^{15}N transfer to the upper forest floor via leaf litter, which was collected in autumn to prevent a ^{15}N flux via litter fall, was less than 5% at the end of experiment. Although this flux is of less importance it may annually be a very important pathway of deposited nitrogen to the forest floor in long term.

Table 8. The concentration of ^{15}N tracer retained (mg g^{-1}), the amount of ^{15}N tracer taken up from soil (mg m^{-2}) and percent recoveries of applied ^{15}N in above- and belowground compartments of the beech seedlings

pool	^{15}N tracer retained				^{15}N tracer recovered (%)	
	^{15}N-NH_4^+		^{15}N-NO_3^-		^{15}N-NH_4^+	^{15}N-NO_3^-
	(mg g^{-1})	(mg m^{-2})	(mg g^{-1})	(mg m^{-2})		
buds	0.031 (0.01)	0.84 (0.32)	0.037 (0.01)	1.22 (0.49)	0.90	1.32
seed leaves	0.006 (0.00)	0.19	0.006 (0.00)	0.15	0.20	0.16
leaf litter (1st season)	0.006 (0.00)	0.22 (0.10)	0.007 (0.00)	0.24 (0.09)	0.23	0.26
leaf litter (2nd season)	0.015(0.00)	0.90 (0.48)	0.013 (0.00)	0.69 (0.31)	0.97	0.75
stems	0.035 (0.01)	6.44 (3.45)	0.041 (0.02)	5.90 (3.38)	6.92	6.40
coarse roots	0.050 (0.01)	4.46 (1.84)	0.065 (0.02)	5.29 (2.95)	4.80	5.75
forest soil fine roots	0.034 (0.02)	0.63 (0.34)	0.037 (0.03)	0.70 (0.61)	0.68	0.76
fine roots in sand layer	0.096 (0.03)	2.25 (1.30)	0.130 (0.03)	3.28 (2.64)	2.42	3.57
plant (total)	0.034 (0.03)	15.9 (4.18)	0.042 (0.04)	17.5 (5.28)	17.1	19.0

Note: Standard deviation is given in parentheses (Values are not statistically significant at $p<0.05$ between tracer N forms)

^{15}N tracer recoveries in plants

Tracer recoveries were highest in stems, where it ranged from 6.92%–6.40% after $^{15}NH_4^+$ and $^{15}NO_3^-$ additions. Coarse roots, total fine roots and leaf litter of the second and first growing seasons exhibited lower values of N tracer recoveries, respectively. The least values for tracer recoveries in beech seedlings were detected in seed leaves, varied between 0.20%–0.16% after $^{15}NH_4^+$ and $^{15}NO_3^-$. The sum of tracers recovered in measured pools ranged from 17.1 to 19.0% of ^{15}N additions, where total percent recoveries were higher after $^{15}NO_3^-$ than after $^{15}NH_4^+$ inputs (Table 9). The results of the percentage recoveries in different plant compartments suggests that the fertilized N taken up by seedlings are assimilated mainly into stems, coarse roots and total fine roots. Although fluxes of tracers into seedlings biomass were small, the fact that ^{15}N recoveries in plant compartments were higher after $^{15}NO_3^-$ than after $^{15}NH_4^+$ additions, suggests that nitrate deposition could have a greater effect on seedlings growth than ammonium deposition. Some tracer studies are consistent with our finding that trees compete better for nitrate than for ammonium inputs. For instance, Nadelhoffer et al. (1999) found total percent recoveries of ^{15}N tracer in the oak and red pine plantations were typically greater after $^{15}NO_3^-$ than after $^{15}NH_4^+$ additions. Studies of cation surplus in beech tree leaves in relation to assimilated nitrogen (Beese 1986) indicated that the beech trees take up mineral nitrogen primarily in the form of nitrate which is then reduced in the leaves. The processes which could have contributed to greater recoveries of tracer after $^{15}NO_3^-$ than after $^{15}NH_4^+$ additions may be the tree species preference for nitrate, or greater competition between tree roots and soil microbes for ammonium (microbial preference for ammonium), or more rapid movement of nitrate to root surfaces (Nadelhoffer et al. 1999). Any or all of these processes may have to some extent contributed to greater recoveries of tracer after $^{15}NO_3^-$ than after $^{15}NH_4^+$ inputs. Comparing percent recoveries of ^{15}N in above- and belowground compartments revealed that most of the ^{15}N taken up by the plants was used to build up the above ground seedlings (53.9%–46.8%), while coarse roots and total fine roots accounted for 28.1%–30.3% and 18.1%–22.8% after $^{15}NH_4^+$ and $^{15}NO_3^-$, respectively.

Conclusion

Nitrogen fertilization had no apparent effect in the partitioning and concentration of carbon and nutrient elements in beech seedlings. Similarly no relationships between the nutrients balances and N input were found in beech seedlings, revealed that fertilization effects may be short-lived so that the balance between biomass production and supply of nutrients is quickly restored to that in unfertilized young trees. Comparing the concentration of ^{15}N tracer retained between the above- and belowground compartments of beech seedlings revealed that fine roots with lower sink strength for ^{15}N can account for a substantial fraction of N-immobilization in plants. Immobilization of deposited ^{15}N by mycorrhizal fine roots may in turn increase the ^{15}N sequestration by their turnover which may be detectable in the soil organic matter. The total amount of ^{15}N tracer retained in seedlings were lower after ^{15}N-ammonium than after ^{15}N-nitrate, suggests that nitrate uptake and deposition could have a greater proportion on seedlings growth than ammonium uptake and deposition.

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